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(54) [Name of Invention] Diminishing agent of toxicity in nerve cells by β-amyloid protein

(57) [Summary]

[Assignment] Inspecting the physiological effect of the ingredients in tea, which is drunk on a daily basis and pose no health problems at all, and developing a substance that has the effect of diminishing the toxicity of β-amyloid protein against nerve cells.

[Resolutions] A diminishing agent of toxicity in nerve cells caused by β-amyloid protein, which includes tea-polyphenol as an active ingredient, and using a method to diminish the toxicity of β-amyloid protein against nerve cells, featuring prescribed effective doses of the diminishing agent defined in Claim 1 to patients whose nerve cells are impinged by the toxicity of β-amyloid protein.

[Scope of claim]

[Claim 1] A diminishing agent of toxicity against nerve cells caused by β-amyloid protein, which includes tea-polyphenol as an active ingredient.

[Claim 2] Diminishing agent of toxicity against nerve cells described in Claim 1, where tea polyphenol is tea catechin and/or theaflavin.

[Claim 3] The diminishing agent of toxicity against nerve cells described in Claim 2, where tea catechin and/or theaflavin is from at least one of the following: (−)−epicatechin gallate (ECG), (−) − epigallocatechin gallate (EGCG), theaflavin, theaflavin-mono-gallate A, theaflavin-mono-gallate B and theaflavin-di-gallate.

[Claim 4] A method to diminish the toxicity of β-amyloid protein against nerve cells, featuring prescribed effective doses of the diminishing agent described in Claim 1 to patients whose nerve cells are impinged by the toxicity of β-amyloid protein.

[Detailed description of the invention]

[0001]

[Technical field of the invention] The present invention relates to the diminishing agent and its effects on the toxicity against nerve cells caused by β-amyloid protein. More in detail, relates to the diminishing agent of toxicity in nerve cells caused by β-amyloid protein, which includes tea-polyphenol as an active ingredient, and the method to diminish toxicity against nerve cells caused by β-amyloid protein by prescribing the diminishing agent to patients whose nerve cells are impinged by the toxicity of β-amyloid protein.

[0002]

[State of technology prior to invention] Recently, dementia has become a social problem in this aging society. This is especially so with Alzheimer's disease, which constitutes 25% of dementia, and senile dementia of Alzheimer type, which attacks younger people, these material diseases as well as cerebrovascular senile dementia form the core of dementia in the older population. These dementias are progressive and are considered extremely hard to cure. Alzheimer's disease and senile dementia of Alzheimer are morphologically degenerative diseases, where the cerebral cortex or hippocampal cells have been shrunken or reduced. Their noticeable pathological findings are (1) a senile plaque in the cerebral cortex or hippocampal cell, and the deposition of β-amyloid protein, and (2) changing the neurofibril.

[0003] The developing mechanism of Alzheimer's disease is widely believed to be attributed to the toxicity of depositional β-amyloid protein against the nerve cells. The β-amyloid protein is a part of the amyloid precursor protein and it is known that it exists across cell membrane perforating domains and extra-cellular domains and shows toxicity against the nerve cells. Therefore, a chemical compound to diminish the toxicity of β-amyloid protein against nerve cells has been searched, and so far, Vitamin E (C. Behl, J.B. Davis, R.Lesleyand D. Schubert, Cell, 77,817-827 (1994)) and (+) (−) catechin (Kunigami, et al., Summary of the lecture at the Japan Agricultural

Chemistry Society in 1996, p53 (1996)) have been found to have a toxicity-diminishing effect.

[0004] On the other hand, the physiological effect of polyphenol, especially tea catechin or theaflavin contained abundantly in tea, has been studied, and it is reported that they have a cholesterol-controlling effect (TokuKaiHei 2-44449 Open Patent Report) and the inhibitory effect of α- amylase (TokuKoHei 3-133928 Open Patent Report) . However, there has been no such report that tea polyphenol has the effect of diminishing the toxicity of β-amyloid protein against nerve cells.

[0005]

[Challenges the invention wants to resolve] The purpose of the present invention is to investigate the physiological effect of the ingredients in tea, which is drunk on a daily basis and poses no health problems at all, to develop a substance that has the effect of diminishing the toxicity of β-amyloid protein against nerve cells, and to develop a substance that has the effect of diminishing toxicity of β-amyloid protein against nerve cells. The inventors of this invention inspected and the existence of a strong diminishing toxicity effect of β-amyloid protein against nerve cells, thus completing this invention.

[0006]

[Measures to solve the challenges] The said invention described in Claim 1 measures the diminishing agent of toxicity against nerve cells caused by β-amyloid protein. The said invention described in Claim 2 measures the diminishing agent of the toxicity against nerve cells of Claim 1, where tea polyphenol is tea catechin and / or theaflavin. The invention described in Claim 3 is a method to diminish the toxicity of β-amyloid protein against nerve cells, featuring prescribed effective doses of the diminishing agent described in Claim 1 to patients whose nerve cells are impinged by the toxicity of β-amyloid protein.

[0007]

[Embodiments of the invention] Tea polyphenol used in this invention is tea catechin shown in Form I below and theaflavin shown in Form II. These may be used either solely or in combination.

[0008]

[Chemistry 1]

[0009] (R_1 shows either H or OH, and R_2 shows H or

[0010]

[Chemistry 2]

Shows [0011]

[0012]

[Chemistry 3]

[0013] (R_3 and R_4 in the form show H or

[0014]

[Chemistry 4]

[0015] , and R_3 and R_4 could be either the same or different.)

[0016] The followings are examples of tea catechin shown in Form I above.

Epicatechin (EC) (R_1=H, R_2=H in Form 1)

Epigallo catechin (EGC) (R_1=OH, R_2=H in Form 1)

Epicatechin gallate (ECg) (R_1=H, R_1=)

[0017]

[Chemistry 5]

[001A] Epigallocatechin gallate (EGCg) (R_1=OH, R_2=)

[0019]

[Chemistry 6]

[0020] Next, examples of theaflavin shown in Form II above are free theaflavin (TF1) (R_3=H, R_4=H in Form II.)

Theaflavin·mono·gallateA(TF2A) (R_3=)

[0021]

[Chemistry7]

[0022] R_4=H)

Theaflavin-mono-gallateB (TF2B) (R_3 =H, R_4= in Form II)

[0023]

[Chemistry 8]

[0024] Theaflavin-di-gallate (TF3) (R_3, R_4 = in Form II)

[0025]

[Chemistry 9]

[0026] The above-mentioned tea polyphenol includes structural properties that can be shown in the same planar structure as in the chemical compound shown in Forms I and II. In the tea catechin shown in Forms I and II, specifically those with 2nd and 3rd configurations of the chemical compound except EC, are cis or trans configuration. Those with 2nd, 3rd, and "2nd and 3rd" configurations of the theaflavin shown in Form II are cis or trans-configuration. All these are used in the present invention. For the activity of tea polyphenol, the active ingredient of the diminishing agent of toxicity against nerve cells in this invention, the density of EC and EGC that shows the strongest activity is 50 μM, the same as (+) -catechin. However, ECg and EGCg have a density of one fifth

of it, and TF1, TF2A, TF2B and "1" F3 at 2/5 of it, showing respectively 5 times and 2.5 times as strong as the activity of (+)−catechin.

[0027] The above-mentioned tea polyphenol contains EGCg and ECg as the main ingredients, and can be produced from tea leaves. Its manufacturing method is described in TokuKaiSho 59-219384 Public Report, TokuKaiSho 60-1370 Public Report, TokuKaiSho 61-130285 Public Report, etc. For example, boil the tea leaves in one of the following solvents: hot water, methanol, ethanol solution, and acetone solution. Dissolve all the extract from the organic solvent, and separate it by dilution from the organic solvent. Furthermore, the concentrate of the extracts that is obtained can be separated into the different substances as mentioned above by using high performance liquid chromatography. Various kinds and forms of tea leaves can be used, such as green tea leaf, non-fermented tea, half-fermented tea, infused tea leaves, instant green tea, or even used tea leaves. Moreover, tea leaves generally contain around 10 % catechin. Theaflavin is contained in black tea, causing its orange color.

[0028] The diminishing agent of toxicity against nerve cells caused by β-amyloid protein in the present invention can be used solely or mixed properly with auxiliary elements for regular use in accordance with the purposes. In other words, it can be mixed properly with diluting agents such as gelatin or sodium alginate, or with solvents such as water or alcohol, or with diluents such as carboxymethyl-cellulose. In the present invention, the diminishing agent of toxicity against nerve cells caused by β-amyloid protein, can be prescribed as oral or non-oral medicine. For example, in the case of oral dosage, it can be taken in various forms, such as with food and drink, or a favorite food such as liquors. They can also be taken with oral hygiene products such as rinse medicine, tooth paste, or troche, and medicine such as internal medicine, powdered medicine, granular medicine, tablet, or capsule, or unregulated drugs. In the case of non-oral prescription, it can be used in liquid form through injections or intravenous drip, or as suppository in solid state or suspension viscous

liquid. For non-oral dosage, topical interstitial administration, hypodermic injection, endodermic injection, intra-muscular injection, intravenous injection as well as external forms of uses such as anal insertion, topical application, and spray, can all be used. The present invention of the diminishing agent of toxicity in nerve cells also can be used as food for livestock.

[0029] The dosage of the said invention of the diminishing agent of toxicity against nerve cells caused by β-amyloid protein can be decided according to its purpose of usage. There is no need to worry about the side effects even if a large amount is given continuously, because the active constituents of this substance are highly safe.

[0030]

[Embodiments] Next, embodiments of the invention will be described in detail. However, the present invention is not limited to these examples.

Embodiment 1

Hippocampal nerve cells of embryos from 18-day pregnant female rats were disseminated on the platos with 96 holes with a cell density of 6×10^4 cell/cm^2. For the culture medium for propagation, a serum-free medium was used, where DMEM medium (product of NISSUI PHARMACEUTICAL CO.,LTD.) was added with 0.5% glucose and GMS-A supplement (Made by GIBCO Inc.) at a rate of one supplement for 1 litre of medium. Dialyzed FCS (blood serum of embryos from cows) (made by JRH BIOSCENCE) was added so as to make the density 10 % from the start for 8-10 hours. Then, it is cultured for 5 days at 37℃ in serum-free medium.

[0031] 5 days after starting the culture microbe, the medium was changed to a serum-free medium with a certain mount of tea polyphenol (tea catechin or theaflavin), and β-amyloid protein (made by BACHEM) was added at a rate of the final density 10 μM. Also, a serum-free medium with only β-amyloid protein added was used in contrast. After cultivating them in these mediums at 37℃ for

24-30 hours, the survival rate of hippocampal nerve cells was measured by using the MTT method (toxicity test using tetrzolium salt)("Manual of Alternative Method of Biological Test" p.51-61 Kyoritu Publishing(1994). Measured value is shown as the number of living cells in the research and control area when the number of living hippocampal nerve cells cultured in the medium with no β-amyloid protein is considered to be 100. The result is shown in Figure 1. In the figure, Aβ(−) indicates an area with no β-amyloid protein added, Aβ(+) indicates a control area with only β-amyloid protein added, EC for a test area with a specified quantity of epicatechin, EGC for a test area with a specified quantity of epigallocatechin, ECg for a test area with a specified quantity of epicatechin gallate, EGCg for a test area with a specified quantity of epigallocatechin gallate, TF1 for a test area with a specified quantity of free theaflavin, TF2 for a test area with a specified quantity of theaflavin-mono-gallate B, and TF3 for a test area with a specified quantity of theaflavin-di-gallate. (+)−C shows a control area with added β-amyloid protein and 50μM of (+)−catechin.

[0032]

[Figure 1]

Figure 1 Survival Rate of Hippocampal nerve cells

Agent name	Quantity of added agent (μM)						(+)−C	Aβ(+)	Aβ(−)
	50	25	20	10	5	1			
EC	118.6						96.0	37.8	100
EGC	107.2	72.8			59.5	53.8	81.0	45.7	100

ECg				96.7			96.0	37.8	100
EGCg			108.2	110.7	77.0	56.5	81.0	45.7	100
TF1			93.1	59.7			88.8	50.9	100
TF2A			109.9	68.3			88.8	50.9	100
TF2B			109.2	63.2			88.8	50.9	100
TFg			118.8	69.0			88.8	50.9	100

[0033] As shown in the table, 100% of hippocampal nerve cells survived in the case of tea catechin, when 50 μM of EC or EGC was added, and 100% hippocampal nerve cells survived when ECg and EGCg, as the main substance of tea catechin, were added by 10 μM. Therefore, it is obvious that gallate type catechin has stronger activity. On the other hand, in the case of theaflavin, 100% of cells survived with 20 μM of either theaflavin although TF1 has slightly weaker activity. Thus, tea polyphenol can reduce or control the toxicity against nerve cells caused by β -amyloid protein with a relatively small addition.

[0034]

[Effect of the invention] This invention, the diminishing agent of toxicity against nerve cells caused by β -amyloid protein, reacts to diminish considerably the toxicity against nerve cells accompanying the deposition of β -amyloid protein. Moreover, it is highly safe because its active constituent is polyphenol, which is a natural product contained in tea and also can be taken by ingestion. As a result, it can be added it to daily food and drink in addition to medical prescriptions.